
Citation:

Lambadarios, E and Zabetakis, I (2002) Does high hydrostatic pressure affect fruit esters? LWT - Food Science and Technology, 35 (4). 362 - 366. ISSN 0023-6438 DOI: <https://doi.org/10.1006/fstl.2001.0863>

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/1753/>

Document Version:

Article (Accepted Version)

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.

Does High Hydrostatic Pressure Affect Fruit Esters?

Evripidis Lambadarios and Ioannis Zabetakis*

University of Leeds, Procter Department of Food Science, Leeds LS2 9JT (U.K.)

Abstract

The effect of high hydrostatic pressure on ester formation and hydrolysis was studied. Six esters and the corresponding carboxylic acids and alcohols were subjected to high-pressure treatments of 400 and 800MPa under three different pH conditions (namely, buffer solutions of pH 4, 6 and 8). The selected compounds were dissolved into buffer solutions, subjected to the pressure treatment and then extracted using dichloromethane. The analysis and quantification were carried out by gas chromatography with flame ionization as detector. High pressure appeared to have no effect on ester formation or hydrolysis under the investigated conditions. In all cases, a small decrease at the levels of carboxylic acids and esters was observed without any evidence of further reaction. This decrease, referred to as decomposition, depended on pressure and pH conditions. Ester decomposition was minimised when a high-pressure treatment of 400MPa in basic conditions (pH 8) was applied. Carboxylic acid decomposition was minimal in basic conditions and it was independent of the pressure applied.

Keywords: strawberry; fruits; esters; high hydrostatic pressure; flavour

Introduction

High hydrostatic pressure (HHP) technology is a novel non-thermal food processing technology where foods are subjected to HHP, generally in the range of 100–600MPa, at or around room temperature (Mertens, 1995). In general, nowadays, food consumers have a high expectation of food quality. There is a strong demand for more ‘natural’ minimally processed, additive free foods and the reported beneficial and unique effects of high pressure appear to offer particular potential in these respects (Johnston, 1994).

Esters are among the most widespread of all naturally occurring compounds. They are moderately polar and thus insoluble in water with dipole moments in the 1.5–2.0D range. Dipole–dipole interactions contribute to the intermolecular attractive forces that cause esters to have boiling points slightly higher than hydrocarbons of similar molecular weight. Because they lack hydroxyl groups, they cannot form hydrogen bonds with each other; consequently, esters have lower boiling points than alcohols of comparable molecular weight (Yamamoto et al., 1991).

Many esters, especially the volatile ones of low molecular weight, have characteristically pleasant odours and are responsible for the flavour and fragrance of many fruits, flowers and artificial flavourings. Generally, the delicacy of natural flavours and fragrances is due to complex mixtures, for instance, more than 100 substances contribute to the flavour of ripe strawberries (Zabetakis & Holden, 1997). On the other hand, cheap artificial flavourings are often single compounds or at the most simple mixtures of esters and ketones.

Among the most common esters contributing to flavour and aroma are: pentyl acetate which resembles the odour of bananas, octyl acetate that of oranges, ethyl butanoate that of pineapples,

pentyl butanoate that of apricot and isopentyl valerate that of apples (McMurry, 1994). Our particular interest lies towards: ethyl and methyl acetate, ethyl and methyl butanoate and ethyl and methyl hexanoate which contribute to the estery and green notes of odour in strawberries. We are also interested in their formation and hydrolysis to carboxylic acids and alcohols (Zabetakis & Holden, 1997; Sumitani et al., 1994; Zabetakis et al., 2000; Larsen et al., 1992).

On a study on the effect of HHP on strawberry flavour, volatile esters were not identified (Zabetakis et al., 2000a). It was suggested that esters were hydrolysed to the respective acids and alcohols during HHP treatment. The absence of esters was explained on the basis of a possible hydrolytic effect that HHP causes (Tauscher, 1995). In this work, we report our results on the effect of HHP on model systems containing fruit esters.

Materials and Methods

All solvents used were of HPLC grade along with all the chemicals that were purchased from Sigma, Gillingham, U.K.

Materials

Buffer solutions. Sodium citrate (58.82 g) was diluted into 950mL of distilled water. The pH of the solution was adjusted by adding citric acid while stirring and a pH meter continuously monitored it. When the solution reached the desired pH value, it was introduced into a 1000mL volumetric flask, which was then filled up to the mark. This sodium citrate–citric acid solution was used to produce buffer solutions of pH 4 and 6. A 0.2 mol/L solution of disodium hydrogen phosphate was prepared by adding 178.05 g of the substance in 1000mL of distilled water. A 0.2 mol/L solution of sodium dihydrogen phosphate was prepared by adding 156.05 g of the substance in 1000mL of distilled water. In 94.70mL of the disodium hydrogen phosphate solution, sodium dihydrogen phosphate solution was gradually added while stirring until the solution reached the desired pH value. The disodium hydrogen phosphate– sodium dihydrogen phosphate solution was used to produce a buffer solution of pH 8.

Solutions of esters, alcohols and carboxylic acids. Three hundred microlitres of the selected ester was introduced into a 10mL volumetric flask and was diluted to 10mL with the appropriate buffer solution (pH varied between 4, 6 and 8). The content of the flask, after vigorous mixing, was divided into two parts (5mL in each part): the control and the sample. The existing esters of the former were extracted three times using 10mL of dichloromethane each time and analysed by GC while the latter was first subjected to pressure and was then extracted three times using 10mL of dichloromethane each time and analysed. In both cases, an internal standard, according to the nature of the analytes, was added just before the extraction so that its final concentration would be 30 mL/mL. In all cases, ethyl butanoate was selected as the internal standard except the case of ethyl butanoate analysis where methyl butanoate was the internal standard.

Three hundred microlitres of the selected carboxylic acid and alcohol were treated the same way as the ester solution. In the solutions of acetic acid and hexanoic acid, butanoic acid was used as the internal standard; while in the case of butanoic acid solutions, acetic acid was the internal standard. In order to estimate the extent of ester formation or hydrolysis in the solutions, the levels of alcohols, acids and esters were analysed by GC.

Sample preparation

High-pressure treatment. The solutions of esters or acids/alcohols were subjected to two different high-pressure treatments: 400 and 800MPa for 15 min at a temperature of 18–22 °C, as described previously (Zabetakis et al., 2000b).

Flavour Analysis. GC analysis. GC analysis was performed using a Fisons 9000 Series Gas Chromatograph. A BP21 (FFAP) column (25m_0.32mm_0.25 mm film thickness), coated with polyethylene glycol ([CH₂–CH₂–O]_n), was used. Injections (0.1 mL) were split-splitless with a split ratio of 30:1. The temperature programme was 30 °C for 4min and later from 30 to 200 °C at 20 °C/min. The carrier gas was helium with a flow rate of 2 mL/min. The detector response was recorded using a Hewlett Packard integrator (HP 3394).

Results and Discussion

The results of this work are presented by comparing the levels of decomposition (i.e. degree of loss) of the analytes (either esters or acids) observed during the various high-pressure treatments. The level of decomposition was calculated by comparing the levels of analytes in the control (i.e. the solution that was not subjected to HHP treatment) and the sample (i.e. the solution that was subjected to HHP treatment). Every experiment was carried out in triplicate and the variation is given in the two tables.

Effect of HHP on esters

In the case of ethyl acetate, the percent decomposition varied between 2 and 14%. The highest degree of decomposition was observed in pH 8 and 800 MPa, while the lowest appears to be in pH 8 and 400MPa conditions. The optimum condition for ethyl acetate is a high-pressure treatment of 400MPa at pH 8. For methyl acetate, the percent decomposition varied between 6 and 10%. The highest degree of decomposition was observed when pressure of 800MPa was applied at pH 6 condition, while the lowest appears to be in pH 8 and 800MPa. As a result, the optimum condition for methyl acetate is a high-pressure treatment of 800MPa at pH 8. In the case of ethyl butanoate, the percent decomposition varied between 0.1 and 7%. The highest degree of decomposition was observed in pH 6 and 800MPa conditions, while the lowest appears to be at pH 4 when a pressure of 400MPa was applied. As a result, the optimum condition for ethyl butanoate is a high-pressure treatment of 400 MPa at pH 4. For methyl butanoate, the percent decomposition varied between 0.1 and 18%. The highest degree of decomposition was observed in pH 6 and 400MPa conditions, while the lowest appears to be at pH 4 when a pressure of 400MPa was applied. As a result, the optimum condition for methyl butanoate is a high-pressure treatment of 400MPa at pH 4. In the case of ethyl hexanoate, the percent decomposition varied between 0.1 and 12%. The highest degree of decomposition was observed in pH 4 and 800MPa conditions, while the lowest appears to be at pH 8 when a pressure of 800MPa was, respectively, applied. As a result, the optimum condition for ethyl hexanoate is a high-pressure treatment of 800MPa at pH 8. For methyl hexanoate, the percent decomposition varied between 0.1% and 18.5%. The highest degree of decomposition was observed in pH 8 and 800MPa conditions, while the lowest appears to be at pH 8 when a pressure of 400MPa was applied. As a result, the optimum condition for methyl hexanoate is a high-pressure treatment of 400MPa at pH 8. These results are summarised in Table 1.

Effect of HHP on acids and alcohols

As far as carboxylic acids and alcohols are concerned, there was no sign of ester formation at all. It was found that there was no ester present after the samples were subjected to high-pressure treatment, while there was only a small decrease in the carboxylic acid concentration further described as decomposition.

In the case of acetic acid–ethanol, the percent decomposition of the acetic acid varied between 0.1 and 8%. The highest degree of decomposition was observed in pH 4 and 800MPa conditions, while the lowest appears to be at pH 8 when a pressure of 800MPa was applied. As a result, the optimum condition, for acetic acid in acetic acid–ethanol solutions is a high-pressure treatment of 800MPa at pH 8. For the mixture of acetic acid–methanol, the percentage decomposition of the acetic acid varied between 0.5 and 6%. The highest degree of decomposition was observed in pH 4 and 800 MPa, while the lowest in pH 6 and 400MPa conditions. As a result, the optimum conditions for acetic acid in acetic acid–methanol solutions are a high-pressure treatment of 400MPa in pH 6. In the case of butanoic acid–ethanol, the percent decomposition of the butanoic acid varied between 0.5 and 5.5%. The highest degree of acid decomposition was observed at pH 4 when the sample was subjected to 800MPa pressure, while the lowest was at pH 8 under 400MPa pressure treatment. As a result, the optimum conditions for butanoic acid in butanoic acid–ethanol solutions are a high-pressure treatment of 400MPa in pH 8. For the mixture of butanoic acid–methanol, the percent decomposition of the butanoic acid varied between 0.5 and 10%. The highest degree of acid decomposition was observed at pH 4 and when the sample was subjected to 800MPa pressure, while the lowest was at pH 8 under 400MPa pressure treatment. As a result, the optimum conditions for butanoic acid in butanoic acid–methanol solutions is a high-pressure treatment of 400MPa in pH 8. In the case of hexanoic acid–ethanol, the percent decomposition of the hexanoic acid varied between 2 and 14%. The highest degree of acid decomposition was observed at pH 4 when the sample was subjected to a 400MPa pressure, while the lowest was found at pH 4 under 800MPa pressure treatment. As a result, the optimum condition for hexanoic acid in hexanoic acid–methanol solutions is a high-pressure treatment of 400MPa in pH 8. For the mixture of hexanoic acid–methanol, the percent decomposition of the acid varied between 0.1 and 19%. The highest degree of acid decomposition was observed at pH 4 and when the sample was subjected to a 400MPa pressure, while the lowest was at pH 6 under 400MPa pressure treatment.

As a result, the optimum condition for hexanoic acid in hexanoic acid–ethanol solutions is a high-pressure treatment of 400MPa in pH 6. In general, the decomposition of hexanoic acid was very low when the acid was subjected to various pressures under different pH conditions. These results are shown in Table 2. A pattern describing the decomposition of the esters and acids cannot be established. In fact, there seems to be an antagonistic effect between two factors when pressure is applied. In the case of high-pressure treatment, a volume decrease is expected to occur when pressure increases. But as far as high-pressure treatment on the overall esterification reaction is concerned, the effect cannot be easily predicted.

In the case of ester solutions, only a small decrease in the ester concentration was observed without any evidence of further reaction. There was no carboxylic acid formation that would indicate ester hydrolysis. Esters did not hydrolyse when pressure was applied because when hydrolysis occurs there is a volume increase. According to the principle of Le Chatelier, when pressure increases a

decrease in volume is expected (McMurry, 1994). Ester hydrolysis leads to the formation of a carboxylic acid and an alcohol, which causes a volume increase. Thus, ester hydrolysis is not favoured by pressure.

On the other hand, solvolytic reactions are enhanced by pressure. Solvolytic reactions are nucleophilic substitutions by the solvent, which, in this case, is water (McMurry, 1994). As a result, the solvent nucleophilically attacks the ester and enhances its hydrolysis. However, the results that we obtained did not show any solvolysis in the system we studied.

The same effect also appears in the case of carboxylic acids and alcohol solutions. Carboxylic acids and alcohols should react to produce esters when pressure is applied due to the oncoming decrease in volume. Moreover, interactions between the acid and the solvent lead to a further increase in volume. The acid interacts with water and hydrates. This hydration leads to a further increase in volume.

On the other hand, hydrogen bonds are formed among the acid, the alcohol and water. The formation of hydrogen bonds can lead to a small volume contraction. Consequently, when pressure is applied, a volume decrease can occur due to hydrogen bond formation and not necessarily due to ester formation.

In any case, the role of pH is very important in ester formation and hydrolysis and thus the selection of the experimental pH conditions is of great importance. In the case of base-induced hydrolysis, a mineral acid, for instance HCl, is needed in the last step to protonate the carboxylate ion and release the carboxylic acid. In the case of acid-catalysed hydrolysis, a protonation is required to activate the carbonyl group during the first step. In both cases, low pH values favour hydrolysis as the degree of protonation increases. As a result, low pH values favour hydrolysis.

In general, it seems that pH has the same effect on both formation and hydrolysis reactions. In low pH values both reactions are favoured, while as pH increases the rate of both reactions is decreased, expecting higher rates of reaction in more acidic conditions.

The obtained results indicate a dependency of decomposition on pH. To be more specific, the degree of decomposition is decreased as pH increases. This is due to the fact that in high pH values the rate of reactions, hydrolysis and formation, is reduced. As a result both ester and acid solutions were more stable in basic conditions. In the case of esters, the optimum condition under which the degree of decomposition minimised and thus appeared to be more stable was a HHP treatment of 400MPa at pH 8. In the case of carboxylic acids, high pressure appeared to have no significant effect on the degree of decomposition. Minimal loss was observed at pH 8, independent of the pressure applied. Acids appeared to be stable at a lower pH (e.g. pH 6) where the decomposition was not affected by pressure. The optimum conditions for esters are 800MPa at pH 8 and for acids are either 400 or 800MPa at pH 8.

Hydrogen bonding and compound volatility are also affected by pH conditions. Hydrogen bonding is affected only in the case of carboxylic acids because esters do not form this type of bonds. In this case, when pH decreases, the degree of acid dissociation also decreases. Thus, [ROOH] becomes the

predominant form. [ROOH] has the ability to form more hydrogen bonds than [ROO₂] species. As a result, acidic conditions enhance hydrogen bond formation.

Ester and carboxylic acid volatility are both affected by pH. In the case of esters, the effect is rather complicated. As pH conditions become more acidic, hydrolysis is enhanced, ROOR₂ concentration is decreased while ROOH concentration is increased. But ROOH species are relatively more volatile than ROOR₂ species. So, even though ester volatility is not decreased, esters are hydrolysed into more volatile acids. A low-pressure treatment in basic conditions is thus required to ensure minimum damage.

On the other hand, pH conditions affect carboxylic acids in the same way as in hydrogen bond formation. In acidic conditions ROOH, which is the predominant species, is more volatile than ROO₂ species. So, as pH decreases, acid volatility is increased.

Here, the reported decomposition (i.e. loss) could be possibly attributed to the high volatility of the compounds used and the inherent experimental error. In all cases, both HHP treatments of 400 and 800MPa had no effect on the overall reaction. The degree of decomposition varied with pressure. Ester decomposition minimised during 400MPa HHP treatment, while acid decomposition appeared to be independent of the pressure applied.

The results reported here could be explained on the basis that high pressure was applied for a short period of time (15 min) and thus it can be considered as a mild treatment. The selection of the processing time was based on the previous work on high-pressure treatment. High pressure was applied for a period of 10–30 min to strawberry jam (Kimura et al., 1994), 15 min to guava juice (Yen & Lin, 1999), 15 min to strawberry (Zabetakis et al., 2000a, b), 10 min to fish and fish products (Ohshima et al., 1993), 10 min to kimchi (Sohn & Lee, 1998) and 10 min to peach (Sumitani et al., 1994). The processing time in our work was selected as an average of the above.

Comparing the obtained results with that of the previous research in this area, 400–600MPa pressure treatments seem to have no or minor effects. A 400MPa treatment on strawberry jam preserved the fresh flavour and natural colour (Kimura et al., 1994), 600MPa sterilised guava juice (Yen & Lin, 1999), 500MPa preserved the original flavour and taste of fish (Ohshima et al., 1993), 400MPa did not affect the structure of kimchi (Sohn & Lee, 1998) and did not change the composition of aromatic compounds in peach (Sumitani et al., 1994). As a result, 400MPa HHP treatment is considered to be a mild process. Here, the reported results are in good agreement with that of the previous work on the effect of HHP on strawberry flavour compounds (Zabetakis et al., 2000a). In that case, highest flavour stability was observed when samples were treated with low pressures of 200–400MPa. In fact, the best flavour retention was observed at 400 MPa.

Acknowledgements

The excellent technical support of Ian Boyes is appreciated.

References

- JOHNSTON, D. E. High-pressure: a new dimension to food processing. *Chemical Industry*, 499–501 (1994)
- KIMURA, K., IDA, M., YOSIDA, Y., OHKI, K., FUKUMOTO, T. AND SAKUI, N. Comparison of keeping quality between pressure-processed jam and heat processed jam. Changes in flavor components, hue and nutrients during storage. *Bioscience Biotechnology and Biochemistry*, 58, 1386–1391 (1994)
- LARSEN, M., POLL, L. AND OLSEN, C. E. Evaluation of the aroma composition of some strawberry (*Fragaria ananassa* Duch) cultivars by use of odour threshold values. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, 195, 536–539 (1992)
- McMurry, J. *Organic Chemistry*, 4th Edn. New York: Brooks– Cole Publishing Company, pp. 250–295 (1994)
- MERTENS, M. Hydrostatic pressure treatment of food: equipment and processing. In: GOULD, G. W. (Ed), *New Methods of Food Preservation*. London: Blackie Academic & Professional, pp. 136–150 (1995)
- OHSHIMA, T., USHIO, H. AND KOIZUMI, C. High pressure processing of fish and fish products. *Trends in Food Science and Technology*, 4, 370–374 (1993)
- SOHN, K. H. AND LEE, H. J. Effects of high-pressure treatment on the quality and storage of kimchi. *International Journal of Food Science and Technology*, 33, 359–365 (1998)
- SUMITANI, H., SUEKANE, S., NAKATANI, A. AND TATSUKA, K. Changes in composition of volatile compounds in high pressure treated peach. *Journal of Agricultural and Food Chemistry*, 42, 785–790 (1994)
- TAUSCHER, B. Pasteurisation of food by hydrostatic high pressure: chemical aspects. *Zeitschrift fur Lebensmittel Untersuchung und Forschung*, 200, 3–13 (1995)
- YAMAMOTO, Y., FURUTA, T., MATSUO, J. AND KURATA, T. Cleavage of esters under nearly neutral conditions at high pressure. Chemo- and Regioselective hydrolysis in organic solvents. *Journal of Organic Chemistry*, 56, 20 (1991)
- YEN, G. C. AND LIN, H. T. Changes in volatile flavour components of guava juice with high-pressure treatment and heat processing and during storage. *Journal of Agricultural and Food Chemistry*, 47, 2082–2087 (1999)
- ZABETAKIS, I. AND HOLDEN, M. A. Strawberry flavour: analysis and biosynthesis. *Journal of the Science of Food and Agriculture*, 74, 412–434 (1997)

ZABETAKIS, I., KOULENTIANOS, A., ORRÚN, O, E. AND BOYES, I. The effect of high pressure on strawberry flavour compounds. Food Chemistry, 71, 51–55 (2000a)

ZABETAKIS, I., LECLERC, D. AND KAJDA, P. The effect of high hydrostatic pressure on the strawberry anthocyanins. Journal of Food and Agriculture Chemistry, 48, 2749–2754 (2000b)

Tables

Table 1 The effect of HHP on ethyl acetate, methyl acetate, ethyl butanoate, methyl butanoate, ethyl hexanoate and methyl hexanoate expressed as percent decomposition of the ester

% Decomposition	pH 4	pH 4	pH 6	pH 6	pH 8	pH 8
	400MPa	800MPa	400MPa	800MPa	400MPa	800MPa
Ethyl acetate	10.1 + ₋ 0.45	7.8 + ₋ 0.42	10.1 + ₋ 0.55	8.2 + ₋ 0.44	2.1 + ₋ 0.11	13.7 + ₋ 0.56
Methyl acetate	7.8 + ₋ 0.39	10.1 + ₋ 0.45	8.2 + ₋ 0.48	10.2 + ₋ 0.51	6.4 + ₋ 0.48	5.8 + ₋ 0.38
Ethyl butanoate	0.1 + ₋ 0.01	3.1 + ₋ 0.11	0.3 + ₋ 0.02	7.5 + ₋ 0.33	3.4 + ₋ 0.23	4.3 + ₋ 0.28
Methyl butanoate	0.1 + ₋ 0.01	14.1 + ₋ 0.43	18.0 + ₋ 0.45	4.1 + ₋ 0.11	4.4 + ₋ 0.23	6.2 + ₋ 0.38
Ethyl hexanoate	1.6 + ₋ 0.12	11.8 + ₋ 0.34	4.4 + ₋ 0.29	5.9 + ₋ 0.44	4.3 + ₋ 0.22	0.1 + ₋ 0.01
Methyl hexanoate	0.9 + ₋ 0.03	2.8 + ₋ 0.11	10.1 + ₋ 0.31	6.2 + ₋ 0.29	0.1 + ₋ 0.01	18.5 + ₋ 0.59

Table 2 The effect of HHP on mixtures of acetic acid/ethanol, acetic acid/methanol, butanoic acid/ethanol, butanoic acid/methanol, hexanoic acid/ethanol and hexanoic acid/methanol expressed as percent decomposition of the acid

	pH 4	pH 4	pH 6	pH 6	pH 8	pH 8
	400MPa	800MPa	400MPa	800MPa	400MPa	800MPa
Acetic acid/ethanol	2.2 + ₋ 0.11	8.2 + ₋ 0.33	4.3 + ₋ 0.28	2.9 + ₋ 0.14	0.41 + ₋ 0.05	0.1 + ₋ 0.01
Acetic acid/methanol	1.2 + ₋ 0.04	5.8 + ₋ 0.29	0.5 + ₋ 0.04	0.7 + ₋ 0.05	5.8 + ₋ 0.23	0.6 + ₋ 0.05
Butanoic acid/ethanol	2.1 + ₋ 0.14	5.5 + ₋ 0.23	1.2 + ₋ 0.14	2.3 + ₋ 0.19	0.5 + ₋ 0.05	0.9 + ₋ 0.07
Butanoic acid/methanol	4.1 + ₋ 0.24	10.1 + ₋ 0.43	2.1 + ₋ 0.14	8.1 + ₋ 0.44	0.5 + ₋ 0.02	7.8 + ₋ 0.38
Hexanoic acid/ethanol	14.1 + ₋ 0.44	2.8 + ₋ 0.34	6.8 + ₋ 0.44	5.5 + ₋ 0.33	2.0 + ₋ 0.11	7.0 + ₋ 0.22
Hexanoic acid/Methanol	19.1 + ₋ 0.55	0.5 + ₋ 0.08	0.1 + ₋ 0.02	0.9 + ₋ 0.06	0.3 + ₋ 0.04	1.5 + ₋ 0.23

+₋